Systematic investigation of the SERS efficiency and SERS hotspots in gas-phase deposited Ag nanoparticle assemblies†

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Gas-phase deposited Ag nanoparticle assemblies are one of the most commonly used plasmonic substrates benefiting from their remarkable advantages such as clean particle surface, tunable particle density, available inter-particle gaps, low-cost and scalable fabrication, and excellent industry compatibility. However, their performance efficiencies are difficult to optimize due to the lack of knowledge of the hotspots inside their structures. We here report a design of delicate rainbow-like Ag nanoparticle assemblies, based on which the hotspots can be revealed through a combinatorial approach. The findings show that the hotspots in gas-phase deposited Ag nanoparticle assemblies are uniquely entangled by the excitation energy and specific inter-particle gaps, differing from the matching conditions in periodic arrays. For Ag nanoparticle assemblies deposited on Formvar-filmed substrates, the mean particle size is maintained around 10 nm, while the particle density can be widely tuned. The one possessing the highest SERS efficiency (under 473 nm excitation) have a particle number density of around 7100 μm⁻². Gaps with an inter-particle spacing of around 3 nm are found to serve as SERS hotspots, and these hotspots contribute to 68% of the overall SERS intensity. For Ag nanoparticle assemblies fabricated on carbon-filmed substrates, the mean particle size can be feasibly tuned. The one possessing the highest SERS efficiency under 473 nm excitation has a particle number density of around 460 μm⁻² and a mean particle size of around 42.1 nm. The construction of Ag–analyte–Ag sandwich-like nanoparticle assemblies by a two-step-deposition method slightly improves the SERS efficiency when the particle number density is low, but suppresses the SERS efficiency when the particle number density is high.

1. Introduction

Gas-phase prepared Ag nanoparticles (G-AgNPs) possess significant advantages such as clean particle surface and tuneable particle size.1–6 Their fabrication processes such as cluster-beam deposition1–7 are low-cost and perfectly compatible with current semiconductor processing technologies. Therefore, G-AgNPs have been deposited onto diverse substrates (including the curved and flexible ones) to fabricate multifarious nanoparticle assemblies for various plasmonic applications such as sensors8–13 and surface-enhanced Raman scattering (SERS).14–17 However, unlike the elaborately designed periodic particle arrays,18–26 G-AgNP deposited assemblies usually have irregular structures with complex nanoparticle configurations closely related to the surface properties of the substrates.3,17 This feature brings great trouble in unveiling the plasmonic couplings among the Ag nanoparticles. As a result, G-AgNP based plasmonic substrates frequently lack structural optimization and represent low performance efficiencies in SERS detection, although they have already been widely used in chemical analysis and biological diagnosis.28,29

As is known, the hotspots located in the interstices among Ag nanoparticle assemblies where the electromagnetic field is enhanced enormously are believed to be the basis of SERS efficiency.30–37 Theoretical simulations based on nanoparticle dimers, trimers and chains indicate that electromagnetic hotspots are usually constructed inside the nanogaps between the neighbouring nanoparticles.14–36,38,39 Generally, the closer the nanoparticles, the stronger the electromagnetic field, as reported in ref. 40. Experimental measurements based on colloidal Ag/Au nanoparticle assemblies...
dimers and few-particle aggregates have demonstrated this conclusion.\textsuperscript{30–36,38,41–43} The nanometer-sized gaps inside the nanoparticle dimers and few-particle aggregates exhibit stupendous activities and even enable detection of SERS signals at the single-molecule level. However, the case of two-dimensional nanoparticle assembly is complicated because the electromagnetic field couplings among the nanoparticles are intricately hybridized. In the pioneering explorations,\textsuperscript{17,20,21,44–50} periodic nanoparticle arrays have been intensely investigated for their simplified structures. Beyond the finding that the surface plasmon resonance (SPR) of the nanoparticle arrays can be modulated to match the excitation wavelength, the role of hotspots in the SERS contribution has also been demonstrated to be critical. As evidenced by Fang et al.\textsuperscript{37} with composite Ag particle arrays, only 63 out of 1,000,000 sites had SERS enhancement factors higher than 10^9, but signals from these hotspots strikingly contributed to around 24% of the overall SERS intensity. Unfortunately, the positions and features of the hotspots could not be revealed, and a subsequent investigation by Chien et al.\textsuperscript{51} using a similar procedure showed that the sites with high SERS enhancements were located in the cracks and grooves, rather differing from the expected tiny interstices between the neighbouring particles. Although many efforts have been made lately, one still cannot confirm where and what the hotspots are in such dense nanoparticle arrays. In comparison to the periodic nanoparticle arrays, the situation of G-AgNP deposited assemblies is much worse due to their complex structures. Since no systematic investigation has been performed so far, the instruction for optimizing their SERS efficiencies is virtually absent. Moreover, questions like whether high-efficient hotspots can be effectively created inside the G-AgNP assemblies, and if so, whether the hotspots will play a dominant role in SERS efficiency are completely unknown.

In order to reveal the hotspots in two-dimensional Ag nanoparticle assemblies as well as their roles in the SERS contribution, exploration of the relationship between nanoparticle configuration and the corresponding SERS performance is of essential significance. The influences of multi-factors including particle size, density, geometry, inter-particle separation and plasmon resonance strength on SERS efficiency should also be carefully verified. Since the G-AgNPs can form diverse nanoparticle assemblies with tuneable particle size and density, the inter-particle gaps can thereby be effectively tuned by controlling the deposition process, even at the scale of a few nanometers.\textsuperscript{2,4} These feasibilities offer an extra degree of freedom to modulate the nanoparticle configurations continuously beyond the limits of conventional top-down fabrication and bottom-up self-assembly techniques. Thereby, well-designed G-AgNP assemblies can open a new path for revealing the hotspots and SERS efficiencies of two-dimensional Ag nanoparticle substrates. From this point of view, we here design multiscale rainbow-like Ag nanoparticle bands (Ag-RNBs) through a shadow-deposition method to perform a combinatorial investigation on their hotspots and corresponding SERS performances. The influences of particle size, number density, inter-particle gaps, SPR wavelength and sandwich-like structure on the SERS efficiencies are also carefully examined. We believe that the findings here can provide some substantial information for the rational design and fabrication of high-efficient plasmonic substrates based on G-AgNP assemblies.

## 2. Experimental

### 2.1 Fabrication of multiscale Ag-RNBs

G-AgNPs were formed by using an ultra-high vacuum cluster source system described elsewhere.\textsuperscript{2} Formvar- or carbon-filmed silica slices and copper grids were used as substrates for Ag nanoparticle deposition. The supporting Formvar or carbon films on silica slices and copper grids were prepared using the same procedure to ensure that they are exactly the same. Masks with different heights were adhered onto the surface of the silica slices. During glancing angle deposition, the shadows of the masks were cast onto the silica slices and copper grids. The regions in the shadows were therefore protected from nanoparticle deposition. The substrates were then mounted onto the substrate holder and put into the deposition chamber. During nanoparticle deposition, the substrate holder was heated to 200 °C and rotated in a stepwise manner with a pre-designed angle step. During the rotation of the substrate holder, the shadow region formed by the masks under the Ag cluster beam changed step by step. The shadow boundary of the high mask moved a longer distance than that of the low mask. In this way, the formed Ag-RNBs in the shadow region of a high mask had a large span of the nanoparticle bands, while those in the low mask led to a small span. The Ag-RNBs fabricated on silica slices with large band spans were used for optical and Raman measurements. The Ag-RNBs fabricated on copper grids with small spans were used for electron microscopic structure characterizations.

### 2.2 Optical and Raman characterizations

The UV-Vis extinction spectra were measured by using a spectrophotometer (Zolix Company) equipped with a homemade microscale light-source. The excitation light was carefully focused onto a spot of size less than 100 μm in diameter. Ag-RNBs were then fixed onto the sample stage and their band-dependent extinction spectra were measured. For Raman measurements, the Ag-RNBs were first immersed into a 10 μm Rhodamine 6G (R6G)-in-methanol solution for 60 s and then taken out vertically and dried under a nitrogen flow. Band-dependent Raman mappings were performed by using an upright-configured confocal Raman microscope (NT-MDT NTEGRA SPECTRA). The excitation lasers were focused through a 100 x objective lens onto a spot with size less than 400 nm in diameter. The laser power used was 100 μW. Raman mappings were performed in sample-scanning mode with a step of 0.5 μm per point and a dwell time of 6 seconds per point. For quantitative estimation of the enhancement factor of the Ag-RNBs, a 100 mM R6G-in-methanol solution was used to prepare a reference sample. Typically, a drop (around 20 μL) of 10 μM R6G solution was dispersed onto a Ag-RNB substrate. For the reference sample, a drop of 100 mM R6G-in-methanol solution was dispersed onto a silica slice. The two samples were then dried under a nitrogen flow. Raman characterization was performed using a 473 nm laser. The laser power used on the
Ag-RNB substrate was 100 μW, while on the silica slice substrate the laser power was 1 mW.

2.3 Microscopic structure and plasmon field mapping

The morphologies of the micro-Ag-RNBs were characterized by using a high resolution transmission electron microscope (TEM) from FEI Company (F20s). The particle size and density of each nanoparticle band were statistically analysed from a number of TEM images by using the Digital Micrograph software. Scanning-TEM (STEM) and electron energy loss spectroscopy (EELS) characterizations were performed by using an aberration-corrected super TEM (FEI Titan Cubed 60–300 kV) equipped with a monochromator. The monochromator was activated to reduce the energy spread of the electron beam from 0.87 eV to 0.17 eV (full width at half maximum). The spectrometer was set to a dispersion of 0.01 eV per pixel and the spectra were recorded on a CCD camera (2048 × 128 pixels). The probing step was 0.64 nm per dwell and the dwell time was 0.2 s. In the data processing stage, the EELS were aligned by using a maximum-intensity alignment algorithm, and the zero-loss peak tail was removed from the summed spectra by using a power law subtraction method. The energy-window-filtered maps were normalized over the full integrated energy by using the Gatan Digital Micrograph system.

2.4 Laser photo-bleaching of R6G molecules

For laser photo-bleaching of R6G molecules, the power of the incident laser (473 nm) used was 1 mW. The intensity of the R6G Raman fingerprints was dynamically monitored in real time with an acquisition time of 1 s. The laser was gradually focused until the Raman intensity started to show a significant decrease. Laser photo-bleaching was performed by sample-scanning of a rectangular region which spanned over all the nanoparticle bands. After that, another scanning was performed over an enlarged rectangular region. This procedure was repeated four times to form multi-stripes with graded photo-bleaching times (see the ESI† for details). The final mapping of the Raman intensity distribution was carried out using a reduced laser power of 100 μW to avoid extra photo-bleaching.

3. Results and discussion

3.1 Design of multiform Ag-RNBs by controllable deposition

Fig. 1(a) shows a schematic illustration of the fabrication of multiscale Ag-RNBs by using an ultra-high vacuum cluster beam deposition system. A collimated G-AgNP beam is formed steadily by using a differential pumping system, and then deposited onto the substrate with a beam shutter to control the deposition mass. On the substrate surface, masks with different heights are placed to form different shadows during glancing angle deposition. As shown in the dotted red inset, with stepwise rotation of the substrate holder, different masks (as marked by h1 and h2) can thereby yield Ag-RNBs with different band spans simultaneously.

Fig. 1(b) shows two typical macro-Ag-RNBs with a span length of around 8 mm fabricated on Formvar- (up) and carbon- (below) filmed silica slices, respectively. Due to the different

![Fig. 1](image-url)

Fig. 1 Morphologies and structures of the fabricated Ag-RNBs. (a) A schematic illustration of the shadow-deposition method. A beam shutter is used to control the deposition mass. The substrate holder is rotated stepwise with a default angle. Different masks are used to form Ag-RNBs with different span lengths. (b) Two macroscale Ag-RNBs with a span length of 8 mm fabricated on Formvar- (up) and carbon- (below) filmed silica slices. (c) Micro-Ag-RNBs fabricated on a Formvar-filmed silica slice with a total span length of around 36 μm. (d) Micro-Ag-RNBs containing eight particle bands with the corresponding TEM images of their nanoparticle structures shown alongside. (e) Band-dependent size distributions of the inter-particle gaps of the micro-Ag-RNBs shown in (d).
particle configurations, the Ag-RNBs show nanoparticle bands with discrepant colours. Fig. 1(c) shows a microscopic image of micro-Ag-RNBs with a span length of around 36 μm. The boundaries between the nanoparticle bands are clear and the structure of each of the nanoparticle bands seems to be very uniform. Fig. 1(d) shows the band-dependent particle configuration of the micro-Ag-RNBs fabricated on a Formvar substrate through TEM characterization. The blank band indicates a narrow region where no nanoparticle is deposited. Bands 1–8 represent nanoparticle assemblies with increasing deposition mass. The deposited G-AgNPs have a log-normal form of size distribution, and their mean particle size, taking into account all the particle bands, is around 10 nm. In contrast, the number density of the micro-Ag-RNBs shows a more close relationship with the deposition mass. The number density increases rapidly at first from band 1 to band 3 and then gradually from band 4 to band 6. The particle number density on band 7 remains almost the same as that on band 6 and then shows a slight decrease on band 8, possibly due to Ostwald ripening and coalescences (see the ESI† for Fig. S1).

In order to quantitatively characterize the particle configurations in the micro-Ag-RNBs, band-dependent distributions of inter-particle separations between each neighbouring particle-pair are statistically calculated by analysing a large number of TEM images taken from each of the nanoparticle bands (see the ESI† for Fig. S2 and S3). As shown in Fig. 1(e), the number of inter-particle gaps having size smaller than 2 nm increases drastically from band 1 to band 8. The number of gaps having sizes of 2–3 nm and 3–4 nm, respectively, represent maxima around bands 5 and 6. The number of gaps having size larger than 4 nm shows a flat slope from band 4 to band 8 and accounts for only a small portion of the total gaps. Gaps with size larger than 6 nm are not calculated since their portion is even lower.

3.2 Band-dependent SERS efficiency of Ag-RNBs fabricated on Formvar-filmed substrates

To estimate the SERS performance of Ag-RNBs fabricated on Formvar-filmed substrates, band-dependent extinction, reflection and Raman mapping are performed after dispersing R6G as a molecular probe. As shown in Fig. 2(a), the Raman fingerprints of R6G molecules (excited by a 473 nm laser) on the Ag-RNBs (band 8, black curve) are remarkably enhanced in comparison to the blank region (red curve). By comparing the Raman intensities measured on the Ag-RNBs and the reference silica slice substrate, the enhancement factors of the highly efficient Ag nanoparticle bands are roughly estimated to be around $3.17 \times 10^7$ to $1.33 \times 10^8$ by assuming that the R6G molecules are homogenously dispersed (see the ESI† for details). Fig. 2(b) shows the intensity mapping by monitoring the average value of the Raman peak at 610 cm$^{-1}$ on the micro-Ag-RNBs. The intensity distribution is clearly band-dependent, showing a terrace-like pattern.
In each nanoparticle band, the obtained intensity is homogenous, demonstrating that R6G molecule adsorption should be uniform. Fig. 2(c) shows the extracted intensity lines from the Raman peaks at 610 cm\(^{-1}\) and 1650 cm\(^{-1}\). It can be seen that the Raman intensities first increase from band 1 to band 5 to approach maximum values and then start to decrease gradually. The confocal reflection mapping synchronously acquired during Raman mapping shown in Fig. 2(d) represents a constant increase from band 1 to band 8, consistent with the increasing deposition mass as designed. Obviously, band 5 is the most SERS efficient nanoparticle band of the Ag-RNBs. In other words, Ag nanoparticle assemblies possessing particle configurations similar to band 5 should have the highest SERS efficiency at an excitation wavelength of 473 nm. Through statistical calculation by TEM, the particle number density is determined to be around 7100 \(\mu m^{-2}\).

By correlating the Raman intensity distributions in Fig. 2(c) with the gap size distributions in Fig. 1(e), it is interestingly found that the gaps having sizes of 2–3 nm and 3–4 nm show a very similar trend to that of Raman intensity. Therefore, it can be inferred that the gaps having size of around 3 nm may serve as SERS hotspots and dominate the SERS efficiency. Noticeably, the measured SRP properties in Fig. 2(e) show that band 5 has a SPR peak at around 520 nm, representing a red-shift from the excitation wavelength of 473 nm. It indicates that the matching condition of the Ag-RNBs between excitation energy and SPR peak is a bit different from that of the periodic particle arrays.

Considering the electromagnetic effect on SERS mechanisms, the enhancement factor can be approximately expressed as \(\frac{E_2}{E_1}E_1^4\), where \(E_2\) and \(E_1\) are field strengths of the local nanoparticles and the excitation laser, respectively.\(^{52,53}\) For simple particle configurations such as dimers, the electromagnetic field strength generally increases with the decrease of the inter-particle separation.\(^{49,54}\) A matched excitation wavelength to the SPR peak thus achieves the maximum electromagnetic enhancement. However, electromagnetic couplings in two-dimensional nanoparticle assemblies are much more complicated. In particular for the Ag-RNBs with randomly dispersed nanoparticles, the SPR response and inter-particle separations are closely related to the particle distributions. With the increase of particle density, the hotspot units may not be exactly the same as the ones contributing to the SPR red-shift. In other words, the Ag nanoparticle assemblies possessing a SPR peak very close to the excitation wavelength of 473 nm may have a low density of activated hotspots. Thus, the SERS efficiency is rather limited by the hotspot density than the SPR strength.

It should be emphasized that SERS hotspots should be discussed at a designated excitation wavelength. A bi-laser (473 nm and 633 nm) Raman mapping on the same micro-Ag-RNBs clearly demonstrates this conclusion (see the ESI† for Fig. S4). In order to unveil the role of SERS hotspots, their contribution to SERS intensity should be precisely estimated. As is known for dye molecules, the molecule decay usually follows \(n(t) = n_0 \exp(-\Gamma t)\), where \(n(t)\), \(n_0\), and \(\Gamma\) are the number of decayed molecules, total number of molecules and decay rate, respectively.\(^{39}\) \(\Gamma\) is known to increase with the increasing local electromagnetic field strength. Thus, the molecules located inside the hotspots where the electromagnetic field is enormously enhanced will be destroyed in advance. According to this instruction, stepwise photo-bleaching is carried out on the micro-Ag-RNBs with seven nanoparticle bands fabricated on a Formvar-filmed substrate. The scanning region is increased step by step with a fixed dwell time of laser irradiation (see the Experimental section and ESI† Fig. S5). Fig. 3(a) shows Raman intensity mapping monitored at 610 cm\(^{-1}\) on the micro-Ag-RNBs after exerting five photo-bleaching times (PBTs). PBT 0 represents the initial intensity distribution on the micro-Ag-RNBs without any photo-bleaching and the maximum intensity appears on band 4. The Raman intensity decreases significantly with increasing PBTs. After the 5 PBTs, the intensity distribution becomes much flat. Fig. 3(b) shows the Raman spectra of R6G molecules acquired on band 4 after each PBT. The fingerprints are well maintained, indicating that only a small portion of R6G molecules have been photo-bleached. With the intensity lines extracted from the stripes of PBT 0 and PBT 5, as shown in Fig. 3(c), it is found that the initial maximum value on band 4 (corresponding to the position at around 21 \(\mu m\)) disappears after photo-bleaching. The flat intensity curve (red line) then shows a similar trend to that of the nanoparticle number density (ESI† Fig. S1). It means that the Raman intensity after photo-bleaching may completely be dominated by the number density of each nanoparticle band. In other words, the R6G molecules located at the sensitive sites are all destroyed and the Raman intensity is mostly contributed by the individual Ag nanoparticles. These sensitive sites should therefore serve as SERS hotspots in the Ag-RNBs. Fig. 3(d) shows the Raman intensity distribution extracted from the white line on band 4. The Raman intensity drops drastically after the first PBT and then decreases slowly due to the reduction of un-decayed R6G molecules in the hotspots. When most of the R6G molecules located in the hotspots have been photo-bleached, the decrease of Raman intensity becomes very slow. By calculating the intensity decrease on band 4, the contribution to SERS intensity from the hotspots is estimated to be 68% (after all five drop steps), while that from the most activated ones is 27% (after the first drop step).

### 3.3 Particle size effect on SERS efficiency

Due to the high diffusivity of Ag atoms on the carbon film, the Ag-RNBs fabricated on carbon-filmed substrates usually show a size-gradient feature. Fig. 4(a) shows the particle configurations of the fabricated Ag-RNBs with ten nanoparticle bands. As can be seen, the Ag nanoparticles grow significantly with the increase of deposition mass due to Ostwald ripening. The size distribution of the Ag nanoparticles becomes more and more divergent from band 4 to band 10. The large ones which grow significantly turn into islands with their geometries becoming irregular. The small ones which seem to be pinned by surface defects and suffer from Ostwald ripening are distributed among these large islands. Fig. 4(b) shows the measured extinction spectra of each nanoparticle band. The SPRs represent a monotonic red-shift with a broadening of resonant peaks. Since the Ag nanoparticles are no longer spherical, the geometry effect and field couplings between them should both generate some influences on the shape of the SPR peaks. The band-dependent Raman
intensities of the Ag-RNBs are measured and shown in Fig. 4(c). It can be seen that band 8 represents the highest SERS efficiency under the excitation of a 473 nm laser. The number density of the nanoparticle band is calculated to be around $460 \text{nm}^2/C_0$ with a mean particle size of around 42.1 nm (without taking into account the pinned clusters smaller than 5 nm). As can be seen in Fig. 4(c), further deposition (in the extra part) will not help improve the SERS performance. This is consistent with the findings that continuous Ag particle films usually possess a low SERS efficiency compared to the well-designed particle assemblies. The irregular particle shapes, inevitable coalescences, uncontrollable inter-particle separations and unpredictable electromagnetic hybridizations may all produce both positive and negative effects on SERS enhancements, leading to a complex domination of the SERS efficiency.

### 3.4 Construction of hotspots by a sandwich-like Ag-RNB matrix

Based on the above-mentioned explorations, it can be concluded that the hotspots in Ag-RNBs play a critical role in SERS efficiency. As is revealed, the hotspots generally require the Ag nanoparticles to locate close to each other so as to create strong coupling field strength. This raises the question of whether hotspots can be constructed by designing multilayer nanoparticle assemblies with analyte molecules as molecular spacers. Hence, sandwich-like micro-Ag-RNBs are designed by a two-step-deposition method to perform an investigation. As shown in Fig. 5(a), micro-Ag-RNBs are first fabricated on a Formvar-filmed substrate following the $X$-direction. R6G molecules are then dispersed onto the micro-Ag-RNBs and dried under a nitrogen flow. At last, a second deposition is performed following a nearly perpendicular $Y$-direction. Fig. 5(b) shows the fabricated micro-Ag-RNBs with sandwich-like structures. The matrix overlapped by the $X$- and $Y$-directions provides Ag nanoparticle assemblies with different densities and configurations. Confocal laser reflection and Raman mapping are performed synchronously on the matrix and shown in Fig. 5(c) and (d). The intensity distribution of the laser reflection clearly reveals the graded diamond squares in the matrix. The regions with heavy deposition exhibit much strong reflection intensities. Interestingly, the Raman intensity mapping in Fig. 5(d) represents inconsistent responses. For the first deposition process along the $X$ direction,
Fig. 5 Fabrication of the matrix of micro-Ag-RNBs with sandwich-like structures and their SERS performances. (a) A schematic illustration of the two-step-deposition method. (b) Microscopic image of the fabricated micro-Ag-RNBs on a Formvar-filmed silica slice. The first deposition is performed in the X direction, while the second deposition follows the Y direction. The dashed black lines indicate the formed matrix of sandwich-like blocks. The dashed red box indicates the survey region for reflection and Raman mappings. (c) Laser reflection mapping synchronously obtained during confocal Raman measurements. (d) Obtained Raman intensity mapping by monitoring the average value of the Raman peak at 610 cm$^{-1}$. (e) Intensity lines extracted from bands 1-y, 2-y and 3-y shown in (d).

Fig. 4 Band-dependent optical and SERS performances on the micro-Ag-RNBs fabricated on a carbon-filmed substrate. (a) Structures and configurations of the Ag nanoparticle bands with increasing deposition mass. (b) Band-dependent extinction spectra of the micro-Ag-RNBs. (c) Band-dependent SERS intensities measured on the micro-Ag-RNBs. The arrow indicates an increasing deposition mass (DM). The extra part on the x-axis indicates much heavier deposition regions.
Raman intensities continuously increase from band 1-y to band 5-y. However, the second deposition along the Y direction produces different influences on each nanoparticle band. By extracting the intensity lines from bands 1-y, 2-y and 3-y, as shown in Fig. 5(e), it is found that Raman intensities are only further enhanced at nanoparticle bands with sparse Ag nanoparticles (i.e. bands 1-y and 2-y). For band 3-y, the second deposition obviously induces a decrease of the Raman intensity. Bands 4-y and 5-y show similar responses to band 3-y, as can be seen in Fig. 5(d).

The findings indicate that the sandwich-like micro-Ag-RNs fabricated by a two-step-deposition method cannot effectively construct a large amount of high-efficient SERS hotspots. Their contributions to SERS efficiency on sparse nanoparticle assemblies are possibly coming from the increased individual Ag nanoparticles. Since it is unable to reveal the locations and spatial configurations of the adsorbed R6G molecules, newly formed nanoparticle gaps by a second deposition may only have a small probability to exactly locate at the molecule sites. Moreover, the quantum tunnelling effect may also play a critical role when the Ag nanoparticles are too close to each other. Thereby, further investigations on their spatial configurations should be performed by a more delicate design.

3.5 STEM-EELS visualization of plasmon field distribution

In order to visualize the field couplings in the Ag-RNs, EELS mapping is performed on a Ag nanoparticle assembly fabricated on a carbon-film-supported copper grid. The sample is plugged into the TEM chamber for several hours to desorb the surface adsorption and then EELS mapping is carried out in the STEM mode. Fig. 6(a) and (f) show the STEM images of the deposited Ag nanoparticles. The white boxes represent the survey regions where EELS mappings are performed. Fig. 6(b) and (g) show the typical EELS spectra of the SPR properties of the Ag nanoparticles. The SPR peaks appear at around 2.9 eV, showing a red-shift of around 0.3–0.4 eV from that of single Ag nanoparticles with a diameter of around 20 nm.55–58 As is known, the SPR properties of individual Ag nanoparticles are size dependent. Their SPR peaks mainly vary from 3.3 eV to 3.4 eV according to the particle size variation from around 20 nm to 5 nm.59 Therefore, the red-shifted SPRs acquired here should be the result of field coupling and hybridization. In order to reveal the dependence of field distribution on excitation energy, a small energy window of 0.2 eV in width is applied to filter the acquired EELS data-cubes. The plasmon field distributions are extracted from Fig. 6(b) and shown in Fig. 6(c) for 2.8–3.0 eV, Fig. 6(d) for 3.0–3.2 eV and Fig. 6(e) for 3.2–3.4 eV, respectively. Fig. 6(h)–(j) extracted from Fig. 6(g) show plasmon field distributions with energy filtering windows of 2.6–2.8 eV, 2.9–3.1 eV and 3.1–3.3 eV, respectively. As can be seen, the sites possessing bright colours indicate positions concentrating strong electromagnetic fields. These sites should serve as hotspots during SERS applications when being strongly excited. For example, in the intensity mapping filtered by the energy window of 2.8–3.0 eV, as shown in Fig. 6(c), the electromagnetic field is concentrated on the lower surfaces of particle 2 and particle 3. When the excitation energy increases, as shown in Fig. 6(e), the gap between particle 3 and particle 4 becomes a new hotspot. Similar results are also
obtained in Fig. 6(h)–(j). The hotspot sites initially formed on the left surface of particle 2 gradually shift to the inter-particle gap between particle 1 and particle 2. Fig. 7 shows the plasmon field mappings from a selected trimer in the nanoparticle assembly. The nanoparticle trimer, as shown in Fig. 7(a), has much smaller inter-particle separations among themselves than against other neighbouring nanoparticles. Thereby, a strong electromagnetic field coupling is expected inside the trimer. Fig. 7(b) and (c) show the summed plasmon spectrum and energy-filtered plasmon field distributions, respectively, extracted from the obtained EELS data-cube. It can be seen that the hot sites are concentrated at the two ends of the trimer when being filtered with a low energy window. With filter windows of increased energy regions, the hot sites are generally located inside the inter-particle gaps.

From the plasmon field distributions, it can be confirmed that the hot sites are co-determined by the excitation energy and the local nanoparticle configurations. The broadened SPR peak of the Ag nanoparticle assembly comprises complex plasmon field couplings related to the local nanoparticle shapes, sizes and configurations. The excited sites concentrating strong plasmon field strengths are probable hotspots during SERS detection. Therefore, in order to optimize the SERS efficiency of the Ag nanoparticle assemblies, the substrate surfaces should be carefully processed in advance. On the other hand, the rational design of integrated matrices of graded Ag nanoparticle assemblies with labelled structures and SRP properties may provide a universal plasmonic substrate for fast SERS detection and analysis.

For Ag-RNBs fabricated on Formvar-filmed substrates, the nanoparticle band possessing the highest SERS efficiency under 473 nm excitation has a particle number density of around \(7 \times 10^4 \text{mum}^{-2}\) with a mean particle size of around 10 nm. The hotspots are found to be the inter-particle gaps having a size of around 3 nm. The SERS intensity contribution from these hotspots is estimated to be 68% of the overall intensity. For Ag-RNBs fabricated on carbon-filmed substrates with a size gradient, the band possessing the highest SERS efficiency has a particle number density of around \(4.6 \times 10^3 \text{mum}^{-2}\), with a mean particle size of around 42.1 nm. Matrices of Ag-RNBs with sandwich-like structures are fabricated by a two-step-deposition method. Raman mapping on the matrices demonstrates that the construction of sandwich-like Ag-RNBs can improve their SERS efficiency when the Ag nanoparticles are sparse. Furthermore, STEM-EELS is used to characterize the energy-filtered plasmon field distributions of the Ag nanoparticle assemblies. The electromagnetic field couplings among the Ag nanoparticles and the possible hotspots are visualized and discussed.

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4. Conclusions
In summary, we have successfully designed multiscale Ag-RNBs on Formvar-filmed and carbon-filmed substrates, respectively. Based on these Ag-RNBs, the SERS efficiency and hotspots are systematically investigated through a combinatorial method.

Notes and references


